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PREVALENCE AND RISK FACTORS FOR ANTIBIOTIC RESISTANCE IN FECAL E. COLI ISOLATED FROM COWS DURING CALVING SEASON IN WESTERN CANADA

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Prevalence and Risk Factors for antimicrobial resistance in fecal *E. coli* isolated from cows during calving season in western Canada.

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Prevalence and Risk Factors for antimicrobial resistance in fecal *E. coli* isolated from cows during calving season in western Canada

Abstract:

The primary study objective was to describe the antimicrobial resistance (AMR) patterns of cows in western Canadian beef herds during calving season, using *E. coli* as an indicator organism. Additional research objectives included: examination of risk factors that contribute to antimicrobial resistance, investigation into the difference in resistance patterns between herds with high and low antimicrobial use, and to gather information to provide greater insight into antimicrobial use and management practices in western Canada. In the spring of 2002 a total of 707 *E.coli* isolates (n=245 cows from 39 farms) were harvested from fresh fecal samples. The antimicrobial susceptibility of a minimum of three *E. coli* isolates per sample was tested using microbroth dilution (Sensititre®, Trek Diagnostic Systems Inc., Clevland, Ohio) and the 2002 National Antimicrobial Monitoring System (NARMS) panel. Data on management practices, animal health, treatment, fecal consistency, and age were also collected. Resistance to at least one antimicrobial was detected in 13.9% (34/245) of the cows and on 46.2% (18/39) of the farms. Multiple drug resistance, defined as resistance to at least two antimicrobials, was seen on 38.5% (15/39) of the farms and in 9.8% (24/245) of the cows. Streptomycin, sulphamethoxazole and tetracycline were the three most common antimicrobials to demonstrate resistance at the isolate, the cow and the herd level. Both isolates (P=0.02) and individual cows (P=0.05) had an increased risk of resistance to multiple antimicrobials if calves in the herd had been treated between 1 and 3 days of age. Additionally, at the isolate level (P=0.02), the cow level (P=0.04) and the herd level (P=0.05) there was an increased risk of resistance to streptomycin if the herd was categorized as a high treatment herd.

Prevalence and Risk Factors for antimicrobial resistance in fecal *E. coli* isolated from cows during calving season in western Canada

Executive Summary:

The Project:

In the summer of 2002, an expert panel advised Health Canada that the use of antimicrobial drugs by Canadian farmers should be significantly reduced and placed under tight controls. Two of the proposed key strategies for controlling antimicrobial resistance (AMR) in food animal agriculture are enhanced surveillance and the development of on-farm interventions. Additionally, guidelines for both prudent use of antimicrobial drugs and critical management practices should be developed and implemented to achieve AMR reduction.

Increasing awareness by producers, veterinarians and agrologists about the presence of antimicrobial resistant *E. coli* will help improve management decisions. By advancing our understanding of AMR in cow-calf herds steps can then be taken to minimize future or growing resistance problems. This will ultimately benefit the industry by minimizing production losses, decreasing environmental contamination with resistant organisms and reducing the risk to humans as a result of infection with resistant pathogens of animal origin. The costs of AMR can be minimized if risk factors are identified and control measures are developed and implemented.

This project was part of a larger strategic research initiative to address the presence of and risk factors for antimicrobial resistance in cow-calf herds. The Western College of Veterinary Medicine, Health Canada and Alberta Agriculture are working together with several Saskatchewan funding agencies to address this important question. In addition to the results of analyses for the cow samples reported in this study, samples have previously been collected from Alberta and Saskatchewan calves at calving, and from calves in the same herds again at weaning during 2002. In 2003 samples were also collected from cow-calf pairs to examine the relationship of resistance in the pair. The preliminary examination of the data from the cow samples collected in the spring of 2002 is presented here.

Results:

Objective 1: To describe *E. coli* antimicrobial resistance patterns in commercial cows at calving.

A minimum of three *E. coli* isolates per sample were tested for sensitivity using the standard 2002 NARMS panels and a micro-broth dilution technique (Sensititre®, TREK Diagnostic Systems Inc., Cleveland, Ohio) (Appendix 1 and 2). The samples were collected from 245 cows on 39 farms for a total of 707 isolates. The majority of the cows were healthy and between 3 and 8 years of age. The fecal samples were scored on a scale of 0 to 3 to identify fecal consistency. A score 0 was assigned if the sample was firm with a score of 3 being assigned to very fluid samples. Of the samples 22.4% (55/245) were scored as 0, 40.8% (100/245) were scored as 1, 32.7% (80/245) were scored as 2 and 1.2% (3/245) were scored as 3, with 2.9% (7/245) of the samples not having a score recorded. The median number of samples collected per herd was 6, with a maximum of 10 per herd and a minimum of 2.

The three antimicrobials to which resistance was most common at the isolate, the cow and the farm level included streptomycin, sulphamethoxazole and tetracycline. Overall for the cows 8.2% (58/707) of the isolates, 13.9% (34/245) of the cows and 46.2% (18/39) of the farms had resistance to at least one antimicrobial. Multiple drug resistance, defined as resistance to greater than 1 antimicrobial, was seen in 6.1% (43/707) of the isolates, 9.8% (24/245) of the cows and 38.5% (15/39) of the farms. The maximum number of antimicrobials to which an individual isolate or cow was resistant was 10. Streptomycin, sulphamethoxazole and tetracycline resistance were identified in the majority of isolates with multiple resistance.

Objective 2: To compare antimicrobial resistance patterns of cows in herds with high antimicrobial use to herds with low antimicrobial use.

Antimicrobial use was assessed at the herd level through the implementation of a standardized questionnaire. The questionnaire summarized the total number of animals in the herd and the number of treatments for both cows and calves. To rank the herds into heavy or light antimicrobial use, the total number of treatments was expressed as a percentage of the total number of animals. The median treatment rate was calculated and used as the cut point for classification of herds into high or low use. Anything less than, the median of 13% was classified as low use and anything above was classified as high use. For the low treatment rate group, the median treatment rate was 5.4% and the mean was 4.9% (95%CI 3.4 to 6.4). For the high treatment rate group, the median treatment rate was 23.7% and the mean was 29.37 (95% CI 19.4 to 39.3).

High or low treatment rate was assessed as a potential risk factor for the all of the following resistance outcomes: resistance to amikacin, amoxicillin/clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, kanamycin, streptomycin, sulphametoxazole, tetracycline, trimethoprim sulphamethoxazole, any antimicrobial and multiple antimicrobials. Analyses were done at each of the isolate, the individual and the herd levels to look at high or low treatment in the herd as a risk factor for all of the above outcomes.

At the isolate (P=0.02), the cow (P=0.04) and the herd (P=0.05) level, streptomycin resistance was more likely in herds classified as a high treatment rate herds. Treatment rate as a category was not a significant risk factor for the other outcomes of interest at any of the three levels investigated.

Objective 3: To determine risk factors that may contribute to the development of antimicrobial resistance.

As in objective 2, antimicrobial use was assessed at the herd level through the implementation of a standardized questionnaire. The questionnaire summarized the total number of animals in the herd and the number of treatments for both cows and calves. Individual treatment records are currently being entered and will be available for future analysis.

Resistance outcomes for the analysis were selected on the basis of the isolate, the cow or the herd demonstrating resistance to that particular antimicrobial. The outcomes of interest included

resistance to amikacin, amoxicillin/clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, kanamycin, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim sulphamethoxazole. Other outcomes considered included resistance to any antimicrobial and resistance to multiple antimicrobials.

Many risk factors were considered in the analysis of this data. Some initial risk factors considered were herd treatment of calves for specific conditions such as scours, pneumonia, and navel ill. Calf treatment was further investigated by looking at treatment of calves according to age and not according to what they were treated for. Calf treatment according to age included the following groupings: calves treated from birth to 24 hours, from 1 to 3 days, from 4 to 13 days and from 2 to 6 weeks. Additionally, treatment of any cows on the farm for specific conditions such as retained placentas, prolapses, pneuomonia, and pinkeye were considered as potentially increasing the risk of resistance development as was overall herd treatment rate of cows. Finally, total herd treatment rate was assessed to see if there was any relationship between it and resistance development.

For the isolate, the individual animal and herd analysis many of the risk factors considered did not illustrate a significant relationship with resistance development. Only, treatment of calves between 1 to 3 days of age increased the chance of multiple drug resistance at the individual (P=0.05) and isolate level (P=0.02).

Objective 4: To gather more knowledge about antimicrobial resistance in cows at calving and antimicrobial management practices commonly used in cow-calf herds at calving.

The standardized questionnaire described earlier was utilized to assess antimicrobial use patterns on the farm. The questionnaire requested information for on farm drug use for cows and calves for various categories of antimicrobials. The major antimicrobial categories included in the questionnaire were, oxytetracylines (Biomycin®, Liquamycin®, etc.), sulpha drugs (Borgal®, Trivetrin®, calf scour boluses, etc.), penicillin, fluoroquinolones (Nuflor®), macrolides (Micotil®), cephalosporin (Excenel®) and others (including Synergistin®).

From the questionnaire it was apparent that the antimicrobials of choice for treating cows and calves on these farms during the calving season were tetracyclines and sulpha drugs. Tetracyclines were used on 71.8% (28/39) of the farms and sulpha drugs were used on 82.1% (32/39) of the farms. Florfenicol (Nuflor®) was next most commonly used medication with 56.4% (22/39) of the farms using it as a treatment. Whereas, only 48.7% (19/39) of the farms used long or short acting penicillins, 38.5% (15/39) of the farms used tilmicosin (Micotil®), and 12.8% of the farms used (5/39) ceftiofur (Excenel®).

On farm drug usage was further broken down to look at how many times each of the drugs were used on farm. Sulpha drugs appear to be the most commonly used drug on cow-calf farms during calving season. A total 7.7% (3/39) of farms used sulpha-based drugs 1 to 3 times, 28.2% (11/39) used them 4 to 10 times and 46.2% (18/39) used them greater than 10 times in the calving season. The second most commonly used antibiotics were oxytetracyclines. Overall 10.3% (4/39) of the farms used oxytetracyclines 1 to 3 times, 23.1% (9/39) 4 to 10 times and 38.5% (15/39) greater than 10 times. Florfenicol (Nuflor®) use was similar to tetracycline use

with 12.8% (5/39) of the farms using florfenicols 1 to 3 times, 25.6% (10/39) 4 to 10 times and 17.9% (7/39) greater than 10 times. Penicillins were used 17.9% (7/39)1 to 3 times, 15.4% (6/39) 4 to 10 times and 15.4% (6/39) greater than 10 times. Tilmicosin (Micotil®) was used on 28.2% (11/39) of the farms 1 to 3 times, 2.6% (1/39) 4 to 10 times and 7.7% (3/39) greater than 10 times. Ceftiofur (Excenel®) was used on 5.1% (2/39) of the farms 1 to 3 times, 2.6% (1/39) 4 to 10 times and 5.1% (2/39) greater than 10 times. Finally, other antimicrobials were used on about 2.6% (1/39) of the herds 1 to 3 times and 7.7% (3/39) greater than 10 times.

The reported drug usage for tetracyclines and sulpha drugs is consistent with the observed pattern of resistance in these cow samples.

Conclusions and Recommendations:

This is the first available surveillance information describing the prevalence of AMR in beef cows at calving in western Canada. Baseline information is required as a first step in any control program. Background information is needed on patterns of antimicrobial resistance to illustrate any variations in these parameters to account for changing production practices and time. The risk factor information collected has the potential for contributing to long-term improvements to environmental health and food safety by minimizing the shedding of resistant organisms by cow-calf herds. Planned analysis of this data and that collected from calves in the spring and fall of 2002 will allow us to look at calves and the changing resistance patterns with age and season. This will substantially increase our ability to identify important risk factors for shedding.

In part because of the relatively low level of resistance identified in the cows and the resulting limited statistical power of the study, very few risk factors were identified for AMR in the cow herd. High treatment rate was associated with streptomycin resistance at the herd, the individual animal and the isolate level. The only other risk factor identified was the association between treatment of calves on the farm between 1 and 3 days of age and individual animal and isolate level multiple drug resistance.

Almost 10% of the cows had at least one isolate with resistance to 2 or more antimicrobials. Multi-resistant organisms are a concern to the animal and human health industry. Baseline research looking at prevalence and risk factors associated with AMR development is necessary to monitor the effects of programs to minimize the development of AMR over time. This information together with that generated from other integrated projects will contribute to our overall knowledge about AMR and will allow us to make more informed decisions on antimicrobial use.

Prevalence and Risk Factors for antimicrobial resistance in fecal *E. coli* isolated from cows during calving season in western Canada.

Technical Report

Project Objectives:

Increasing antimicrobial resistance (AMR) poses a growing threat to human and animal health. Physicians, veterinarians and producers are under increasing scrutiny due to concerns about inappropriate or excessive use of antimicrobials. There is growing pressure for more restrictive regulations on antimicrobials. In order to ensure that effective antibiotics are available for use in livestock, antimicrobial use practices are being examined.

There is no published information on antimicrobial use and AMR patterns in cow-calf herds from western Canada. Strategies for control begin with development of baseline information on the prevalence and risk factors associated with AMR.

In the fall of 2002 Health Canada released a report from an Advisory Committee addressing Animal Uses of Antimicrobials and the impact on resistance and Human Health. The report suggested that the use of antimicrobials by Canadian farmers should be significantly reduced and placed under tight controls. Specific recommendations of the report include: that antimicrobials for agriculture use should be obtained only by prescription, and that the rise of antimicrobial resistance in farm animals should be monitored by on-farm surveillance programs. The current project was developed as part of a research plan to identify the prevalence and risk factors associated with antimicrobial use (AMU) in cow-calf herds. Knowledge gained through this project will provide baseline information on the occurrence of AMR, and the risk factors associated with its development. This information will contribute to the design of control strategies to minimize the further development of resistance.

The specific objectives of the study were to:

1) To describe *E. coli* antimicrobial resistance patterns in commercial cows at calving.
2) To compare antimicrobial resistance patterns of cows in herds with heavy antimicrobial use to herds with low antimicrobial use.
3) To determine the risk factors that may contribute to the development of antimicrobial resistance.
4) To gather more knowledge about antimicrobial resistance in cows at calving and antimicrobial management practices commonly used in cow-calf herds.

Background :

• What is the impact of antimicrobial resistance on animal agriculture?

In June, 2002 Health Canada released a report on "Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health." The document contained about 38 recommendations on how antimicrobial use could be modified to help minimize the impact of developing resistance. Specific recommendations of the report included: that antibiotics for agricultural use should be obtained only by prescription, that the use of antibiotics by farmers and ranchers should be monitored, and that the rise of antibiotic resistance in farm animals should be monitored. The advice given to Health Canada was that the use of antibiotics by Canadian farmers should be significantly reduced and placed under tighter controls.

This is not a new issue and the industry has recognized for some time that antimicrobial resistance issues could possibly affect animal agriculture in many ways. For instance, the process of approving antibiotics for potential use in food animals is now under review. Interest groups are also calling for a ban to the practice of using the same antibiotics that are currently (or prospectively) used in human medicine for subtherapeutic use or as growth promotants in food animals.

The use of antimicrobial drugs in animal agriculture is essential for improving animal health and welfare through disease treatment, carcass quality, and economic efficiency of growth and production. If the industry loses efficacious antibiotics because of resistance development or limiting availability, the consequences and costs to the industry would be substantial.

• What factors contribute to antimicrobial resistance?

The development of a resistant microorganism and its subsequent transmission in human or animal populations is often a multi-factorial and multi-step process. The problem of antimicrobial resistance in human pathogens is increasing. The human population's changing age structure, an increase in high-use institutional care environments, and patients' demands for antimicrobial treatment of viral infections all contribute to this serious problem.

• What are the public's main concerns about antimicrobial resistance?

People are concerned that antimicrobial use in food animals can lead to the selection of enteric organisms that are antimicrobial-resistant and, in some cases, zoonotic. These organisms could eventually be transferred to people who consume contaminated food or are in direct contact with animals. Scientists have described examples of indirect or direct resistance transfer from animals to humans, but no one knows the true magnitude of the medical impact from antimicrobial resistance in livestock.

Another concern is the potential reservoir of resistant, environmental bacteria found in the feces of animals that are fed antimicrobials.

• How can antimicrobial resistance be managed?

Actions to manage the development of antimicrobial resistance must be based on sound science. Two of the proposed key strategies for reducing antimicrobial resistance in food animal agriculture are enhanced surveillance and the development of on-farm interventions. Those proposed interventions include prudent anti-microbial use and critical management practices.

• What do scientists need to learn about the development of antimicrobial resistance in livestock?

Most of the current antibiotic-resistance research is focused on intensive livestock operations such as feedlots. However, some calves arrive at the feedlot shedding resistant bacteria. Research is necessary to understand the origin of this resistance.

Prior to this study, we had no information about *E. coli* antibiotic resistance in cow-calf pairs during the period following calving when the stocking density, stress, and antimicrobial use in the herd may be at its highest. By studying commercial cow-calf herds that have different management practices, different levels of disease and different levels of antibiotic use, we can begin to look at possible emerging patterns of resistance. Then we can consider what can be done to minimize further development.

There is very little useful information to guide producers and policy makers in western Canada. Some researchers have discussed the development of antibiotic resistance in asymptomatic and diarrheic calves. Most of this research has been done in dairies. None of the research has been done during calving or weaning in commercial cow-calf herds similar to those found in Saskatchewan. Research articles from Japan, England, United States, India, Austria, Spain, Finland, Denmark, and Canada have described variations of antibiotic resistance and pathogenic *E. coli* in cattle. Currently available research comes from feedlots, dairies, laboratory submissions, swine operations, and the poultry industry.

• How will an understanding of resistance patterns help the cattle industry?

By implementing the Canadian Cattle Identification Agency (CCIA) tags, the industry now has the ability to track animals from the farm gate to the time of slaughter. This system increases awareness of what is happening at each stage of beef production. Through its "Quality Starts Here" program, the Canadian Cattlemen Association has also worked to identify critical control points to ensure the production of a safe and reliable product.

The mandate of the "Hazard Analysis and Critical Control Points" (HACCP) quality assurance program is to reduce the risk of biological, physical and chemical contamination of beef. The control of antibiotic resistance in cow-calf herds is an important part of on-farm food safety initiatives.

By gaining an understanding of resistance patterns, the cow-calf industry will be capable of identifying critical management practices that are potentially contributing to the problem. In turn, this knowledge will enable the industry to minimize the problem by modifying critical management practices.

Materials and Methods:

Fecal samples were collected in the spring of 2002 from cows during the calving season. The fecal samples were cultured for *E. coli*, and researchers characterized the antimicrobial resistance pattern by describing the minimum inhibitory concentrations for a series of isolates from each sample. The study compared the occurrence of antibiotic resistance between herds with high antibiotic use during spring calving and herds that had low antibiotic use during the same period.

Experimental Design

In the spring of 2002 a total of 707 isolates (n=248 cows from 44 farms) were harvested from fresh cow fecal samples. Herds included in this study were selected from a larger on going health and productivity study. Based on herd treatment records, the research team randomly assigned cow-calf operations to either the top 25th percentile for antimicrobial use, representing moderate to high antimicrobial use or the bottom 25th percentile, for low to no antimicrobial use. Fresh fecal samples were collected from up to 10 cows per herd. Using *E. coli* as an indicator organism to test for antimicrobial susceptibility, a minimum of three *E. coli* isolates per sample were tested using microbroth dilution (Sensititre®, TREK Diagnostic Systems Inc., Cleveland, Ohio) and the 2002 National Antimicrobial Monitoring System (NARMS) panel. Data on management practices, animal health, treatment, fecal consistency, and age were also collected.

Statistical Analysis

Descriptive statistics were generated using a commercial software program (SPSS 11.0, SPSS Inc., Chicago, Illinois). Associations between the occurrence of resistance to individual antimicrobials, multiple antimicrobials, and any antimicrobials at the isolate, individual animal level, and herd level and various risk factors were analyzed using a generalized estimating equations (GEE) method to account for clustering within herd. Data were analyzed using a commercial statistical software program (SAS v.8.2 for Windows (PROC GENMOD); SAS Institute, Cary, North Carolina, USA). Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to herd, and an exchangeable correlation structure. Variables will remain in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis.

Results:

<u>*Objective 1*</u>: To describe *E. coli* antimicrobial resistance patterns in commercial cows at calving.

A minimum of three *E. coli* isolates per sample were tested for sensitivity using the standard 2002 NARMS panels and a micro-broth dilution technique (Sensititre®, TREK Diagnostic Systems Inc., Cleveland, Ohio) (Appendix 1 and 2). The samples were collected from 245 cows

on 39 farms for a total of 707 isolates. The majority of the cows were healthy and between 3 and 8 years of age (Tables 1 and 2). The fecal samples were scored on a scale of 0 to 3 to identify fecal consistency. A score 0 was used where the sample was firm, 1 when the sample was pasty, 2 when the sample was loose and 3 when the sample was watery. Of the samples 22.4% (55/245) were scored as 0, 40.8% (100/245) were scored as 1, 32.7% (80/245) were scored as 2 and 1.2% (3/245) were scored as 3, with 2.9% (7/245) of the samples not having a score recorded (Table 3). The median number of samples collected per herd was 6, with a maximum of 10 per herd and a minimum of 2.

The three antimicrobials to which resistance was most commonly identified at the isolate, the cow and the farm level included streptomycin, sulphamethoxazole and tetracycline (Table 4). Resistance to at least one antimicrobial was identified in 8.2% (58/707) of the isolates, 13.9% (34/245) of the cows and 46.2% (18/39) of the farms. Multiple drug resistance, defined as resistance to greater than 1 antimicrobial, was seen in 6.1% (43/707) of the isolates (Table 5), 9.8% (24/245) of the cows (Table 6) and 38.5% (15/39) of the farms. The maximum number of antimicrobials to which an individual isolate or animal was resistant was 10. Streptomycin, sulphamethoxazole and tetracycline resistance patterns were identified in the majority of isolates with multiple resistance (Table 7).

Table 1: Distribution of Cow Age Classes within the Sample Population

Animal Age	*Age Code*	*Frequency*	*Percent*
<= 2	0	44	18.0
3-5	1	101	41.2
6-8	2	60	24.5
9-11	3	25	10.2
12-14	4	15	6.1
Total		245	100.0

Table 2: Reported Cow Health Status for Sample Population

Health Status	*Frequency*	*Percent*
Not Recorded	1	.4
No	1	.4
Yes	243	99.2
Total	245	100.0

Table 3: Recorded Consistency Scores for Fecal Samples Collected from Cows

Fecal Score	*Frequency*	*Percent*
0	55	22.4
1	100	40.8
2	80	32.7
3	3	1.2
Not recorded	7	2.9
Total	245	100.0

Table 4: Summary of resistance data for *E. coli* from cow fecal samples summarized at the isolate, individual and farm level

Antimicrobial	*Number of Isolates Resistant*	*Isolate Resistant %*	*Number of Cows Resistant*	*Individual Resistant %*	*Number of Farms Resistant*	*Farm Resistance %*
Amikacin	3 / 707	0.42	3 /245	1.2	3 / 39	7.7
Amoxicillin/ Clavulanic Acid	1 / 707	0.14	1/ 245	0.4	1 / 39	2.6
Ampicillin	3 / 707	0.42	2 / 245	0.8	2 / 39	5.1
Ceftiofur	1 / 707	0.14	1 / 245	0.40	1 / 39	2.6
Cephalothin	1 / 707	0.14	1 / 245	0.40	1 / 39	2.6
Chloramphenicol	6 / 707	0.85	4 / 245	1.6	4 / 39	7.7
Kanamycin	3 / 707	0.42	3 / 245	1.2	3 / 39	7.7
Streptomycin	31 / 707	4.4	19 / 245	7.7	19 / 39	30.8
Sulfamethoxzale	35 / 707	5.0	20 / 245	8.1	20 / 39	30.8
Tetracycline	57 / 707	8.1	30 / 245	12.1	30 / 39	43.6
TMPS	4 / 707	0.57	4 / 245	1.6	4 / 39	7.7

Table 5: Frequency of Multiple Resistance in Isolates from Cow Fecal Samples*

Number of Isolates Resistant		*Total number of Isolates*					
58	of	707	=	**8.2%**	resistant to at least 1 antimicrobial		
43	of	707	=	**6.1%**	resistant to at least 2 antimicrobials		
27	of	707	=	**3.8%**	resistant to at least 3 antimicrobials		
8	of	707	=	**1.1%**	resistant to at least 4 antimicrobials		
1	of	707	=	**0.1%**	resistant to at least 5 antimicrobials		
1	of	707	=	**0.1%**	resistant to at least 6 antimicrobials		
1	of	707	=	**0.1%**	resistant to at least 7 antimicrobials		
1	of	707	=	**0.1%**	resistant to at least 8 antimicrobials		
1	of	707	=	**0.1%**	resistant to at least 9 antimicrobials		
1	of	707	=	**0.1%**	resistant to at least 10 antimicrobials		
15	of	707	=	**2.1%**	resistant to	**1**	antimicrobial
16	of	707	=	**2.3%**	resistant to	**2**	antimicrobials
19	of	707	=	**2.7%**	resistant to	**3**	antimicrobials
7	of	707	=	**1.0%**	resistant to	**4**	antimicrobials
0	of	707	=	**0.0%**	resistant to	**5**	antimicrobials
0	of	707	=	**0.0%**	resistant to	**6**	antimicrobials
0	of	707	=	**0.0%**	resistant to	**7**	antimicrobials
0	of	707	=	**0.0%**	resistant to	**8**	antimicrobials
0	of	707	=	**0.0%**	resistant to	**9**	antimicrobials
1	of	707	=	**0.1%**	resistant to	**10**	antimicrobials

* Individual isolate is resistant to 2 or more antimicrobials

Table 6: Frequency of Cows Identified with Isolates Having Multiple Resistance*

Resistant or Not	*Frequency*	*Percent*
No Resistance	221	90.2
Resistant	24	9.8
Total	245	100.0

* Individual isolate is resistant to 2 or more antimicrobials

Table 7: Antimicrobial Resistance Patterns Identified in *E.coli* isolates from Cow Fecal Samples

Count of # Isolates/AMR Pattern											
# of Resistant Antimicrobials per Isolate	Total										
0	649										
0 Total	649										
1	15									TET	
1 Total	15										
2	8								SMX	TET	
	2		AMP							TET	
	6							STR		TET	
2 Total	16										
3	17							STR	SMX	TET	
	1					CHL			SMX		SXT
	1								SMX	TET	SXT
3 Total	19										
4	4					CHL		STR	SMX	TET	
	2						KAN	STR	SMX	TET	
	1							STR	SMX	TET	SXT
4 Total	7										
10	1	AMC	AMP	FUR	CEF	CHL	KAN	STR	SMX	TET	SXT
10 Total	1										
Grand Total	707										

Objective 2: To compare antimicrobial resistance patterns of cows in herds with high antimicrobial use to herds with low antimicrobial use.

Antimicrobial use was assessed at the herd level through the implementation of a standardized questionnaire. The questionnaire summarized the total number of animals in the herd and the number of treatments for both cows and calves. To rank the herds into heavy or light antimicrobial use, the total number of treatments was expressed as a percentage of the total number of animals. The median treatment rate was calculated and used as the cut point for classification of herds into high or low use. Anything less than the median of 13% was classified as low use and anything above was classified as high use. After dividing the herds into high or low usage a total of 48.7% (19/39) ranked as low use (treatment rate <13%) and 51.3% (20/39) as high use (treatment rate >13%). For the low treatment rate group, the median treatment rate was 5.4% and the mean was 4.9% (95%CI 3.4 to 6.4). For the high treatment rate group, the median treatment rate was 23.7% and the mean was 29.4 (95% CI 19.4 to 39.3).

High or low treatment rate was assessed as a potential risk factor for the all of the following resistance outcomes: resistance to amikacin, amoxicillin/clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, kanamycin, streptomycin, sulphametoxazole, tetracycline, trimethoprim sulphamethoxazole, any antimicrobial and multiple antimicrobials. Three separate analyses were done at the isolate, the individual and the herd level to look at high or low treatment in the herd as a risk factor for all of the above outcomes.

At the isolate (P=0.020), the cow (P=0.04) and the herd (P=0.05) level, streptomycin resistance was more likely in herds classified as a high treatment rate herds. Treatment rates were not a significant risk factor for the other outcomes of interest at any of the three levels investigated.

Objective 3: To determine risk factors that may contribute to the development of antimicrobial resistance.

As in objective 2, antimicrobial use was assessed at the herd level through the implementation of a standardized questionnaire. The questionnaire summarized the total number of animals in the herd and the number of treatments for both cows and calves. Individual treatment records are currently being entered and will be available for future analysis.

Resistance outcomes for the analysis were selected on the basis of the isolate, the cow or the herd demonstrating resistance to that particular antimicrobial. The outcomes of interest included resistance to amikacin, amoxicillin/clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, kanamycin, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim sulphamethoxazole. Other outcomes considered included resistance to any antimicrobial and resistance to multiple antimicrobials.

Many risk factors were considered in the analysis of this data. Some initial risk factors considered were herd treatment of calves for specific conditions such as scours, pneumonia, and navel ill. Calf treatment was further investigated by looking at treatment of calves according to age and not according to what they were treated for. Calf treatment according to age included the following groupings: calves treated from birth to 24 hours, from 1 to 3 days, from 4 to 13

days and from 2 to 6 weeks. Additionally, treatment of any cows on the farm for specific conditions such as retained placentas, prolapses, pneuomonia, and pinkeye were considered as potentially increasing the risk of resistance development, as was overall herd treatment rate of cows. Finally, total herd treatment rate was assessed to see if there was any relationship between it and resistance development.

For the isolate, the individual animal and herd analysis many of the risk factors considered did not illustrate a significant relationship with resistance development. Only, treatment of calves between 1 to 3 days of age increased the chance of multiple drug resistance at the individual (P=0.05) and isolate level (P=0.02).

Objective 4: To gather more knowledge about antimicrobial resistance in cows at calving and antimicrobial management practices commonly used in cow-calf herds at calving.

The standardized questionnaire described earlier was utilized to assess antimicrobial use patterns on the farm. The questionnaire requested information about on farm drug use for cows and calves for various categories of antimicrobials. The major antimicrobial categories included in the questionnaire were, oxytetracylines (Biomycin®, Liquamycin®, etc.), sulpha drugs (Borgal®, Trivetrin®, calf scour boluses, etc.), penicillin, fluoroquinolones (Nuflor®), macrolides (Micotil®), cephalosporin (Excenel®) and others (including Synergistin®).

From the questionnaire it was apparent that the antimicrobials of choice for treating cows and calves on these farms during the calving season were tetracyclines and sulpha drugs. Tetracyclines were used on 71.8% (28/39) of the farms and sulpha drugs were used on 82.1% (32/39) of the farms. Florfenicol (Nuflor®) was next most commonly used medication with 56.4% (22/39) of the farms using it as a treatment. Whereas, only 48.7% (19/39) of the farms used long or short acting penicillins, 38.5% (15/39) of the farms used tilmicosin (Micotil®), and 12.8% of the farms used (5/39) ceftiofur (Excenel®).

On farm drug usage was further broken down to look at how many times each of the drugs were used on farm. Sulpha drugs appear to be the most commonly used drug on cow-calf farms during calving season. A total 7.7% (3/39) of farms used sulpha-based drugs 1 to 3 times, 28.2% (11/39) used them 4 to 10 times and 46.2% (18/39) used them greater than 10 times in the calving season. The second most commonly used antibiotics were oxytetracyclines. Overall 10.3% (4/39) of the farms used oxytetracyclines 1 to 3 times, 23.1% (9/39) 4 to 10 times and 38.5% (15/39) greater than 10 times. Florfenicol (Nuflor®) use was similar to tetracycline use with 12.8% (5/39) of the farms using florfenicols 1 to 3 times, 25.6% (10/39) 4 to 10 times and 17.9% (7/39) greater than 10 times. Penicillins were used 17.9% (7/39)1 to 3 times, 15.4% (6/39) 4 to 10 times and 15.4% (6/39) greater than 10 times. Tilmicosin (Micotil®) was used on 28.2% (11/39) of the farms 1 to 3 times, 2.6% (1/39) 4 to 10 times and 7.7% (3/39) greater than 10 times. Ceftiofur (Excenel®) was used on 5.1% (2/39) of the farms 1 to 3 times, 2.6% (1/39) 4 to 10 times and 5.1% (2/39) greater than 10 times. Finally, other antimicrobials were used on about 2.6% (1/39) of the herds 1 to 3 times and 7.7% (3/39) greater than 10 times.

A list of the participating herds along with the categorization of use of the major antimicrobials considered is summarized in Table 8. This provides a quick over view of how many times each drug was used on that particular farm for treatment of either cows or calves. From this

breakdown of on farm usage it is not surprising to see that tetracyclines and sulpha drugs demonstrate the most resistance in these cow samples

In the next table the number of cows resistant to each antimicrobial is listed by herd along with the overall treatment rate (Table 9). Additionally, this table provides the total number of samples collected, the number of resistant animals and the total number of antimicrobials that have resistance on each farm. Interestingly, some of the herds with the highest treatment rates have the either no or low numbers of resistant animals. This is goes against what we have found in some of our other studies and what expected to find here. The reason for this may because the resistance found in the cows is at a fairly low prevalence compared with the resistance found in their calves and we may have needed more samples per farm to measure herd level prevalence reliably.

Along with considering which drugs herd owners are using for treatment, it is interesting to consider the reasons that farms treated either cows or calves. The questionnaire looked at farm treatment of calves for navel ill, pneumonia and scours. Scours came out on top with 76.9% (30/39) of the farms treating calves with scours (Table 10). The second most common calf aliment reported on farms was pneumonia with 56.4% (22/39) of farms treating for it (Table 11). Finally, navel ill was the next most common condition with 43.6% (17/39) of the farms reporting treatment (Table 12).

Cows were treated most commonly for retained placentas with reports from 56.4% (22/39) of the farms (Table 13), followed by uterine prolapses on 20.5% (8/39) of the farms (Table14). Vaginal prolapses and pneumonia in cows were treated on 17.9% (7/39) of the farms (Table 15 and 16) and finally cows were treated for pinkeye on 15.4% (6/39) of the farms (Table 17).

Table 8: Summary of Farm Antimicrobial Use

Farm	*Oxytetracycline*	*Sulpha drugs*	*Penicillin*	*Florfenicol*	*Tilmicosin*	*Cefitiofur*	*Other*
1	3	3	2	3	0	3	0
2	3	3	2	3	0	0	3
3	3	3	0	3	1	0	0
4	2	3	2	2	0	0	0
5	3	0	0	3	0	0	0
6	0	2	1	2	1	0	0
7	3	3	3	2	1	0	0
8	0	3	0	2	0	0	0
9	2	2	3	2	1	0	1
10	2	3	1	1	0	1	0
11	3	3	1	1	0	1	0
12	0	2	2	2	3	0	0
13	3	2	0	3	3	2	0
14	3	3	0	1	0	0	0
15	1	0	0	2	1	0	0
16	2	0	0	1	1	0	0
17	3	3	0	0	0	0	0
18	3	1	3	0	1	0	0
19	0	2	1	0	2	0	0
20	2	1	0	0	1	0	0
21	0	0	1	0	0	0	0
22	3	3	1	0	3	0	0
23	0	2	2	0	0	0	0
24	1	2	0	2	0	0	0
25	2	0	0	0	0	0	0
26	0	3	0	0	0	0	0
27	3	3	0	0	0	3	3
28	0	0	1	0	0	0	0
29	2	3	2	0	1	0	0
30	0	2	0	3	0	0	0
31	0	0	0	1	0	0	0
32	2	3	0	0	0	0	0
33	3	2	0	2	0	0	0
34	0	1	0	0	0	0	0
35	1	3	3	0	0	0	0
36	3	3	0	0	1	0	0
37	3	2	0	0	0	0	0
38	1	3	3	2	0	0	3
39	2	2	3	3	1	0	0

Code for Antimicrobial use on farm:

0 = No use of that antimicrobial on farm
1 = Used the antimicrobial 1 to 3 times
2 = Used the antimicrobial 4 to 10 times
3 = Used the antimicrobial >10 times

Table 9: Farm level summary of treatment rate and antimicrobial resistance in cows

Farm	AMK	AMC	AMP	FUR	CEF	CHL	KAN	STR	SMX	TET	STX	Overall treatment rate (%)	# of cow samples	# of cows with resistant isolates	# of antibiotics to which resistance was seen
1	0	0	0	0	0	0	0	0	0	0	0	28	6	0	0
2	0	0	0	0	0	0	0	0	0	0	0	36	6	0	0
3	0	0	0	0	0	0	0	0	0	0	0	26	4	0	0
4	0	0	0	0	0	0	0	2	2	2	0	18	9	2	3
5	0	1	1	1	1	1	1	1	1	2	1	14	9	2	10
6	0	0	0	0	0	0	0	0	0	0	0	6	6	0	0
7	0	0	0	0	0	0	0	0	0	0	0	59	6	0	0
8	0	0	0	0	0	0	0	1	0	1	0	16	6	1	2
9	0	0	0	0	0	0	0	1	1	1	0	17	6	1	3
10	0	0	0	0	0	0	0	0	0	0	0	5	6	0	0
11	1	0	0	0	0	0	0	1	0	1	0	17	6	2	3
12	0	0	0	0	0	0	0	0	1	1	0	8	2	1	2
13	0	0	1	0	0	2	0	3	3	4	0	25	9	4	5
14	0	0	0	0	0	0	0	0	0	0	0	104	6	0	0
15	0	0	0	0	0	0	0	0	0	0	0	9	6	0	0
16	0	0	0	0	0	0	0	0	0	0	0	2	6	0	0
17	0	0	0	0	0	0	0	0	0	0	0	7	6	0	0
18	0	0	0	0	0	0	0	0	0	0	0	9	9	0	0
19	0	0	0	0	0	0	0	0	0	0	0	8	9	0	0
20	0	0	0	0	0	0	0	0	0	0	0	7	7	0	0
21	0	0	0	0	0	0	0	0	0	0	0	1	7	0	0
22	0	0	0	0	0	0	0	0	0	0	0	25	5	0	0
23	0	0	0	0	0	0	0	1	0	1	0	13	6	1	2
24	0	0	0	0	0	0	0	0	0	0	0	5	6	0	0
25	1	0	0	0	0	0	1	1	1	1	0	2	8	2	5
26	0	0	0	0	0	0	0	0	0	1	0	14	8	1	1
27	0	0	0	0	0	0	1	1	1	1	0	2	6	1	4
28	0	0	0	0	0	0	0	0	1	3	0	2	10	3	2
29	0	0	0	0	0	0	0	0	0	0	0	19	5	0	0
30	0	0	0	0	0	0	0	1	1	1	1	0	2	1	4
31	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0
32	0	0	0	0	0	0	0	0	0	0	0	16	6	0	0
33	0	0	0	0	0	1	0	4	5	4	2	23	5	5	5
34	1	0	0	0	0	0	0	0	0	0	0	4	5	1	1
35	0	0	0	0	0	0	0	0	0	0	0	5	6	0	0
36	0	0	0	0	0	0	0	2	2	3	0	35	8	3	3
37	0	0	0	0	0	0	0	0	0	2	0	11	6	2	1
38	0	0	0	0	0	0	0	0	1	1	0	47	6	1	2
39	0	0	0	0	0	0	0	0	0	0	0	35	6	0	0
Total	**3**	**1**	**2**	**1**	**1**	**4**	**3**	**19**	**20**	**30**	**4**		**245**	**34**	

Table 10: Proportion of Farms Reporting Treatment of Calf Scours

Use	*Frequency*	*Percent*
No	9	23.1
Yes	30	76.9
Total	39	100.0

Table 11: Proportion of Farms Reporting Treatment of Calf Pneumonia

Use	*Frequency*	*Percent*
No	17	43.6
Yes	22	56.4
Total	39	100.0

Table 12: Proportion of Farms Reporting Treatment of Calf Navel Ill

Use	*Frequency*	*Percent*
No	22	56.4
Yes	17	43.6
Total	39	100.0

Table 13: Proportion of Farms Reporting Treatment of Cow Retained Placenta's

Use	*Frequency*	*Percent*
No	17	43.6
Yes	22	56.4
Total	39	100.0

Table 14: Proportion of Farms Reporting Treatment of Cow Uterine Prolapse

Use	*Frequency*	*Percent*
No	31	79.5
Yes	8	20.5
Total	39	100.0

Table 15: Proportion of Farms Reporting Treatment of Cow Vaginal Prolapse

Use	*Frequency*	*Percent*
No	32	82.1
Yes	7	17.9
Total	39	100.0

Table 16: Proportion of Farms Reporting Treatment of Cow Pneumonia

Use	*Frequency*	*Percent*
No	32	82.1
Yes	7	17.9
Total	39	100.0

Table 17: Proportion of Farms Reporting Treatment of Cow Pinkeye

Use	*Frequency*	*Percent*
No	33	84.6
Yes	6	15.4
Total	39	100.0

Conclusions and Recommendations:

This is the first available surveillance information describing the prevalence of AMR in western Canadian beef cows at calving. Baseline information is required as a first step in any control program. Background information is needed on patterns of antimicrobial resistance to illustrate any variations in these parameters to account for changing production practices and time. The risk factor information collected has the potential for contributing to long-term improvements to environmental health and food safety by minimizing the shedding of resistant organisms by cow-calf herds. Planned analysis of this data and that collected from the calves in the spring and fall of 2002 will help in the understanding of changing resistance patterns with age and season. This will substantially increase our ability to identify important risk factors for shedding. Through identification of animals within the herd that are most likely to shed resistant organisms preventative policies and techniques could be implemented. By analysing the shedding patterns, control measures such as treatment restrictions could be utilized to their fullest potential.

In part because of the relatively low level of resistance identified in the cows and the resulting limited statistical power of the study, very few risk factors were identified for AMR in the cow herd. High treatment rate was associated with resistance to streptomycin at the herd, the individual animal and the isolate level. The only other risk factor identified was treatment of calves on the farm between 1 and 3 days of age which seemed to contribute to individual animal and isolate level multiple drug resistance.

Almost 10% of the cows had at least one isolate with resistance to 2 or more antimicrobials. Multi-resistant organisms are a concern to the animal and human health industry. Baseline research looking at prevalence and risk factors associated with AMR development is necessary to monitor the effects of programs to minimize the development of AMR over time. This project in co-ordination with the other projects mentioned earlier will contribute to our overall knowledge about AMR and will allow us to make more informed decisions on antimicrobial use.

Project Activities, Achievements, Presentations:

Results will be disseminated at producer meetings as opportunities become available. Veterinary organizations will also be targeted potentially including the Alberta and Saskatchewan Veterinary Medical Associations and the Western Canadian Association of Bovine Practitioners. Information and recommendations will be published in lay and technical journals. Lay publications may include the Western Producer, or Cattleman's. The technical journals would include publications like the Canadian Veterinary Journal.

Future Development:

The project was very successful in generating other related research which will extend our understanding of antimicrobial resistance. With other available funding, about another 800 cow isolates and a number of spring cow-calf pair samples were examined from Alberta and Saskatchewan herds, to look for additional risk factors that may contribute to the development of resistance. In addition we have already also completed testing a large number of samples collected in the spring and fall from beef calves. The occurrence of AMR in the spring-born

calves is approximately 10-fold higher than that identified in the cows near calving and the calves sampled the subsequent fall. This finding has led to the need for additional research to follow individual calves from birth until past weaning to evaluate this finding. Additionally, we are having a number of samples genotyped to allow us to start gathering antimicrobial genetic information on resistance in cow-calf herds.

Beneficial Information Generated:

We have developed the first estimates of the prevalence of AMR in beef cows from western Canada at calving. Some initial information on risk factors for shedding has also been generated. When combined with information from the larger data set, we hope to be able to produce some more specific recommendations for management and control.

The proposed study has provided several potential benefits to the cattle industry in western Canada. It has provided valuable baseline data on the prevalence of antimicrobial resistance. This information allows us to determine how the cow-calf industry compares to other areas of animal agriculture, and to determine the effectiveness of any future control programs. Additionally, *E. coli* is an important cause of food-related illness in humans and a leading cause of disease and production losses in cow-calf herds. By examining antimicrobial resistance that focuses on cow-calf herds, the cattle industry has illustrated that it has taken steps demonstrate its concern about the issue to the general public. The study has also provided the industry with some preliminary potential risk factors. Furthermore, the collected risk factor information will potentially contribute to long-term improvements to minimize the development of future antibiotic resistance. Finally, the study will provide veterinarians and producers with current information about antibiotic resistance in cow-calf herds that will allow them to improve treatment of sick animals and to minimize the development of antimicrobial resistance.

References:

Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health. Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health, Health Canada, Health Products and Food Branch, June 2002

Appendix 1: INTERPRETATION POINTS

Amikacin	Amoxicillin/Clavulanic Acid	Ampicillin	Apramycin	Cefoxitin	Ceftiofur	Ceftriaxone	Cephalothin	Chloramphenicol	Ciprofloxacin	Gentamicin	Imipenem	Kanamycin	Nalidixic Acid	Streptomycin	Sulphamethoxazole	Tetracycline	Trimethoprim/Sulphamethoxazole
AMK	AMC	AMP	APR	FOX	FUR	CRO	CEF	CHL	CIP	GEN	IPM	KAN	NAL	STR	SMX	TET	SXT
32	16	16	16	16	4	32	16	16	2	8	8	32	16	64	256	8	2

These are the "interpretation points" (1 dilution below NCCLS*) used for the analysis

NCCLS=National Committee on Clinical Laboratory Standards

CMV7CNCD Plate Exceptions:

1. Amikacin results > 4 will be labeled not interpretable because the breakpoint is 4 dilutions beyond the range of this panel.
2. Streptomycin breakpoint was set at 64ug/ml
3. Apramycin and Imipenem are not available on this panel

Appendix 2: BREAKPOINTS AND INTERPRETATION POINTS

Antimicrobials	BP	Range (ug/ml)	Interpretation Point
Amikacin	>= 64	0.5-4	**4**
Amoxicillin/ Clavulanic Acid	>= 32/16	1-32	**16**
Ampicillin	>= 32	1-32	**16**
Apramycin	N/A	N/A	
Cefoxitin	>= 32	0.5-16	**16**
Ceftiofur	>= 8	0.12-8	**4**
Ceftriaxone	>= 64	0.25-64	**32**
Cephalothin	>= 32	2-32	**16**
Chloramphenicol	>= 32	2-32	**16**
Ciprofloxacin	>= 4	0.015-4	**2**
Gentamicin	>= 16	0.25-16	**8**
Imipenem	N/A	N/A	
Kanamycin	>= 64	8-64	**32**
Nalidixic Acid	>= 32	0.5-32	**16**
Streptomycin	None	32-64	**32**
Sulfamethoxazole	>= 512	16-512	**256**
Tetracycline	>= 16	4-32	**8**
Trimethoprim/ Sulphamethoxazole	>= 4/76	0.12-4	**2**

Break points are the point at which the concentration of the antibiotic no longer is able to prevent growth of the bacteria. It is the point that is used to identify resistance because all bacteria growth that is not inhibited at this point is classified as resistant.